

CLAIMS

1. A polypeptide having the ability to bind CEA comprising the amino acid sequence:

Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys (SEQ ID NO:110),

wherein:

X₄ is Asn, Glu, Asp, or Met;

X₅ is Leu, Phe, Tyr, Trp, Val, Met, Ile, or Asn;

X₆ is Phe, Leu, Asp, Glu, Ala, Ile, Lys, Asn, Ser, Val, Trp, Tyr, Gly, or Thr;

X₇ is Lys, Phe, Asp, Gly, Leu, Asn, Trp, Ala, Gln, or Thr;

X₈ is Asn, Pro, Phe, Gly, Asp, Ala, Ser, Glu, Gln, Trp, His, Arg, Met, Val, or Leu;

X₉ is Gln, Lys, Leu, or Gly;

X₁₀ is Trp, Ala, or Tyr; and

X₁₁ is Phe, Thr, Met, Ser, Ala, Asn, Val, His, Ile, Pro, Trp, Tyr, Gly, Leu, or Glu.

2. A polypeptide having the ability to bind CEA comprising the amino acid sequence:

X₁-X₂-X₃-Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys-X₁₂-X₁₃-X₁₄ (SEQ ID NO:111),

wherein:

X₁ is Asp, Asn, Ala, or Ile;

X₂ is Trp;

X₃ is Val, Ile, Met, Tyr, Phe, Pro, or Asp;

X₄ is Asn, Glu, Asp, or Met;

X₅ is Leu, Phe, Tyr, Trp, Val, Met, Ile, or Asn;

X₆ is Phe, Leu, Asp, Glu, Ala, Ile, Lys, Asn, Ser, Val, Trp, Tyr, Gly, or Thr;

X₇ is Lys, Phe, Asp, Gly, Leu, Asn, Trp, Ala, Gln, or Thr;

X₈ is Asn, Pro, Phe, Gly, Asp, Ala, Ser, Glu, Gln, Trp, His, Arg, Met, Val, or Leu;

X₉ is Gln, Lys, Leu, or Gly;

X₁₀ is Trp, Ala, or Tyr;

X₁₁ is Phe, Thr, Met, Ser, Ala, Asn, Val, His, Ile, Pro, Trp, Tyr, Gly, Leu, or Glu;

X₁₂ is Asn, Asp, Glu, Pro, Gln, Ser, Phe, or Val;

X₁₃ is Val, Leu, Ile, Pro, Ala, Gln, Ser, Met, Glu, Thr, Lys, Trp, or Arg; and

X₁₄ is Leu, Met, Val, Tyr, Ala, Ile, Trp, His, Pro, Gln, Glu, Phe, Lys, Arg, or Ser.

3. A polypeptide having the ability to bind CEA comprising the amino acid sequence:

Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys (SEQ ID NO:3),

wherein:

X₄ is Asn, Glu, or Met;
X₅ is Asn, Leu, Met or Phe;
X₆ is Asp, Gly, Ile, Lys Phe or Thr;
X₇ is Ala, Gln, Gly, Lys or Thr;
X₈ is Arg, Asn, Asp, Glu or Gly;
X₉ is Gln, Gly or Leu;
X₁₀ is Ala, Trp or Tyr;
X₁₁ is Ala, Gly, His, Phe, Thr or Val;

4. The polypeptide according to Claim 3, wherein:

X₄ is Glu;
X₅ is Asn, Leu, Met or Phe;
X₆ is Asp, Gly, Ile, Lys, Phe or Thr;
X₇ is Lys;
X₈ is Arg, Asn, Asp, Glu or Gly;
X₉ is Gln;
X₁₀ is Trp; and
X₁₁ is Ala, Gly, His, Phe, Thr or Val.

5. The polypeptide according to Claim 3, comprising the amino acid sequence:

X₁-X₂-X₃-Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys-X₁₂-X₁₃-X₁₄ (SEQ ID NO:1),

wherein:

X₁ is Asn or Asp;
X₂ is Trp;
X₃ is Asp, Phe or Val;
X₄ is Asn, Glu or Met;
X₅ is Asn, Leu, Met or Phe;

X₆ is Asp, Gly, Ile, Lys, Phe or Thr;
 X₇ is Ala, Gln, Gly, Lys or Thr;
 X₈ is Arg, Asn, Asp, Glu or Gly;
 X₉ is Gln, Gly or Leu;
 X₁₀ is Ala, Trp or Tyr;
 X₁₁ is Ala, Gly, His, Phe, Thr or Val;
 X₁₂ is Asn, Gln, Phe, Ser or Val;
 X₁₃ is Arg, Leu, Pro or Ser; and
 X₁₄ is Leu, Ser, Trp or Tyr.

6. The polypeptide according to Claim 5, having the amino acid sequence:

X₁-Trp-Val-Cys-Glu-X₅-X₆-Lys-X₈-Gln-Trp-X₁₁-Cys-Asn-X₁₃-X₁₄
 (SEQ ID NO:2), wherein:

X₁ is Asn or Asp;
 X₅ is Asn, Leu, Met or Phe;
 X₆ is Asp, Gly, Ile, Lys, Phe or Thr;
 X₈ is Arg, Asn, Asp, Glu or Gly;
 X₁₁ is Ala, Gly, His, Phe, Thr or Val;
 X₁₃ is Arg, Leu, Pro or Ser; and
 X₁₄ is Leu or Tyr.

7. The polypeptide according to Claim 5, comprising an amino acid sequence selected from the group consisting of:

Asn-Trp-Val-Cys-Asn-Leu-Phe-Lys-Asn-Gln-Trp-Phe-Cys-Asn-Ser-Tyr
 (SEQ ID NO:4);

Asp-Trp-Val-Cys-Glu-Asn-Lys-Lys-Asp-Gln-Trp-Thr-Cys-Asn-Leu-Leu
 (SEQ ID NO:5);

Asn-Trp-Asp-Cys-Met-Phe-Gly-Ala-Glu-Gly-Trp-Ala-Cys-Ser-Pro-Trp
 (SEQ ID NO:6);

Asp-Trp-Val-Cys-Glu-Lys-Thr-Thr-Gly-Gly-Tyr-Val-Cys-Gln-Pro-Leu
 (SEQ ID NO:7);

Asn-Trp-Phe-Cys-Glu-Met-Ile-Gly-Arg-Gln-Trp-Gly-Cys-Val-Pro-Ser

(SEQ ID NO:8); and

Asp-Trp-Val-Cys-Asn-Phe-Asp-Gln-Gly-Leu-Ala-His-Cys-Phe-Pro-Ser
(SEQ ID NO:9).

8. A polypeptide having the ability to bind CEA comprising the amino acid sequence:

X₁-X₂-X₃-Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys-X₁₂-X₁₃-X₁₄ (SEQ ID NO:1),

wherein:

X₁ is Asp, Asn, Ala, or Ile;

X₂ is Trp;

X₃ is Val, Ile, Met, Tyr, Phe, Pro, or Asp;

X₄ is Asn, Glu, or Asp;

X₅ is Leu, Phe, Tyr, Trp, Val, Met, Ile, or Asn;

X₆ is Phe, Leu, Asp, Glu, Ala, Ile, Lys, Asn, Ser, Val, Trp, or Tyr;

X₇ is Lys, Phe, Asp, Gly, Leu, Asn, or Trp;

X₈ is Asn, Pro, Phe, Gly, Asp, Ala, Ser, Glu, Gln, or Trp;

X₉ is Gln, or Lys;

X₁₀ is Trp;

X₁₁ is Phe, Thr, Met, Ser, Ala, Asn, Val, His, Ile, Pro, Trp, or Tyr;

X₁₂ is Asn, Asp, Glu, Pro, Gln, or Ser;

X₁₃ is Val, Leu, Ile, Pro, Ala, Gln, Ser, Met, Glu, Thr, Lys, or Trp; and

X₁₄ is Leu, Met, Val, Tyr, Ala, Ile, Trp, His, Pro, Gln, Glu, Phe, Lys, or Arg.

9. The polypeptide of claim 1, wherein:

X₄ is Asn, or Glu;

X₅ is Leu, Phe, Tyr, Trp, or Ile;

X₆ is Phe, Leu, Asp, Glu, Ile, Ser, Val, or Gly;

X₇ is Lys;

X₈ is Asn, Pro, Gly, Asp, Ala, Ser, His, Met, Val, or Leu;

X₉ is Gln;

X₁₀ is Trp;

X₁₁ is Phe, Thr, Ser, Ala, Asn, Val, His, Ile, Trp, Tyr, Leu, or Glu;

10. The polypeptide of claim 2, wherein:

X₁ is Asp, or Asn;
X₂ is Trp;
X₃ is Val, Ile, or Met;
X₄ is Asn, or Glu;
X₅ is Leu, Phe, Tyr, Trp, or Ile;
X₆ is Phe, Leu, Asp, Glu, Ile, Ser, Val, or Gly;
X₇ is Lys;
X₈ is Asn, Pro, Gly, Asp, Ala, Ser, His, Met, Val, or Leu;
X₉ is Gln;
X₁₀ is Trp;
X₁₁ is Phe, Thr, Ser, Ala, Asn, Val, His, Ile, Trp, Tyr, Leu, or Glu;
X₁₂ is Asn, or Asp;
X₁₃ is Val, Leu, Ile, Pro, Ala, Gln, Ser, or Met,; and
X₁₄ is Leu, Met, Val, Tyr, Trp, His, Gln, Arg, or Ser.

11. The polypeptide according to Claim 2, comprising an amino acid sequence as depicted in Tables 5, 6, 8, and 9 (SEQ ID NOs:37-109 and 113-151).

12. The polypeptide according to Claim 1, 2, 3, 5, 8, 9, or 10, wherein said polypeptide binds to CEA but does not bind to NCA.

13. The polypeptide according to Claim 1, 2, 3, 5, 8, 9, or 10, wherein said polypeptide has a K_d for CEA which less than 7 μM.

14. A method of detecting CEA in a subject comprising the steps of:

- a) detectably labeling a polypeptide according to any one of Claims 1-11;
- b) administering to said subject the labeled polypeptide; and, thereafter,
- c) detecting the labeled polypeptide in the subject.

15. The method according to Claim 14, wherein said polypeptide is labeled with a radioactive compound.
16. The method according to Claim 15, wherein said radioactive compound includes indium.
17. The method according to Claim 15, wherein said radioactive compound includes technetium.
18. The method according to Claim 14, wherein said detecting step is indicative of colon cancer, breast cancer, lung cancer, cervical cancer, ovarian cancer, stomach cancer, bladder cancer, pancreatic cancer or esophageal cancer.
19. A method of treating a CEA associated disease comprising the step of: administering to a subject in need of treatment for such a disease a composition comprising a polypeptide according to any one of Claims 1-11 conjugated with a therapeutic agent effective for treating said disease.
20. The method according to Claim 19, wherein said CEA associated disease is colon cancer, breast cancer, lung cancer, cervical cancer, ovarian cancer, stomach cancer, bladder cancer, pancreatic cancer or esophageal cancer.
21. The method according to Claim 19, wherein said therapeutic agent is a radioactive agent.
22. The method according to Claim 19, wherein said therapeutic agent is a chemotherapeutic agent.
23. The method according to Claim 19, wherein said therapeutic agent is a toxin or enzyme.
24. A recombinant bacteriophage expressing exogenous DNA encoding a CEA binding polypeptide having an amino acid sequence comprising:
- Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys (SEQ ID NO:110),
- wherein:
- X₄ is Asn, Glu, Asp, or Met;
- X₅ is Leu, Phe, Tyr, Trp, Val, Met, Ile, or Asn;

X₆ is Phe, Leu, Asp, Glu, Ala, Ile, Lys, Asn, Ser, Val, Trp, Tyr, Gly, or Thr;
 X₇ is Lys, Phe, Asp, Gly, Leu, Asn, Trp, Ala, Gln, or Thr;
 X₈ is Asn, Pro, Phe, Gly, Asp, Ala, Ser, Glu, Gln, Trp, His, Arg, Met, Val, or Leu;
 X₉ is Gln, Lys, Leu, or Gly;
 X₁₀ is Trp, Ala, or Tyr; and
 X₁₁ is Phe, Thr, Met, Ser, Ala, Asn, Val, His, Ile, Pro, Trp, Tyr, Gly, Leu, or Glu.

and wherein said binding polypeptide is displayed on the surface of said bacteriophage.

25. A recombinant bacteriophage expressing exogenous DNA encoding a CEA binding polypeptide having an amino acid sequence comprising:

X₁-X₂-X₃-Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys-X₁₂-X₁₃-X₁₄ (SEQ ID NO:111),

wherein:

X₁ is Asp, Asn, Ala, or Ile;
 X₂ is Trp;
 X₃ is Val, Ile, Met, Tyr, Phe, Pro, or Asp;
 X₄ is Asn, Glu, Asp, or Met;
 X₅ is Leu, Phe, Tyr, Trp, Val, Met, Ile, or Asn;
 X₆ is Phe, Leu, Asp, Glu, Ala, Ile, Lys, Asn, Ser, Val, Trp, Tyr, Gly, or Thr;
 X₇ is Lys, Phe, Asp, Gly, Leu, Asn, Trp, Ala, Gln, or Thr;
 X₈ is Asn, Pro, Phe, Gly, Asp, Ala, Ser, Glu, Gln, Trp, His, Arg, Met, Val, or Leu;
 X₉ is Gln, Lys, Leu, or Gly;
 X₁₀ is Trp, Ala, or Tyr;
 X₁₁ is Phe, Thr, Met, Ser, Ala, Asn, Val, His, Ile, Pro, Trp, Tyr, Gly, Leu, or Glu;
 X₁₂ is Asn, Asp, Glu, Pro, Gln, Ser, Phe, or Val;
 X₁₃ is Val, Leu, Ile, Pro, Ala, Gln, Ser, Met, Glu, Thr, Lys, Trp, or Arg; and
 X₁₄ is Leu, Met, Val, Tyr, Ala, Ile, Trp, His, Pro, Gln, Glu, Phe, Lys, Arg, or Ser.

and wherein said binding polypeptide is displayed on the surface of said bacteriophage.

26. A recombinant bacteriophage expressing exogenous DNA encoding a CEA binding polypeptide having an amino acid sequence comprising:

X₁-X₂-X₃-Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys-X₁₂-X₁₃-X₁₄ (SEQ ID NO:1),

wherein

X₁ is Asp, Asn, Ala, or Ile;

X₂ is Trp;

X₃ is Val, Ile, Met, Tyr, Phe, Pro, or Asp;

X₄ is Asn, Glu, or Asp;

X₅ is Leu, Phe, Tyr, Trp, Val, Met, Ile, or Asn;

X₆ is Phe, Leu, Asp, Glu, Ala, Ile, Lys, Asn, Ser, Val, Trp, or Tyr;

X₇ is Lys, Phe, Asp, Gly, Leu, Asn, or Trp;

X₈ is Asn, Pro, Phe, Gly, Asp, Ala, Ser, Glu, Gln, or Trp;

X₉ is Gln, or Lys;

X₁₀ is Trp;

X₁₁ is Phe, Thr, Met, Ser, Ala, Asn, Val, His, Ile, Pro, Trp, or Tyr;

X₁₂ is Asn, Asp, Glu, Pro, Gln, or Ser;

X₁₃ is Val, Leu, Ile, Pro, Ala, Gln, Ser, Met, Glu, Thr, Lys, or Trp; and

X₁₄ is Leu, Met, Val, Tyr, Ala, Ile, Trp, His, Pro, Gln, Glu, Phe, Lys, or Arg

and wherein said binding polypeptide is displayed on the surface of said bacteriophage.

27. A recombinant bacteriophage expressing exogenous DNA encoding a CEA binding polypeptide having an amino acid sequence comprising:

Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys (SEQ ID NO:110),

wherein:

X₄ is Asn, or Glu;

X₅ is Leu, Phe, Tyr, Trp, or Ile;

X₆ is Phe, Leu, Asp, Glu, Ile, Ser, Val, or Gly;

X₇ is Lys;

X₈ is Asn, Pro, Gly, Asp, Ala, Ser, His, Met, Val, or Leu;

X₉ is Gln;

X₁₀ is Trp;

X₁₁ is Phe, Thr, Ser, Ala, Asn, Val, His, Ile, Trp, Tyr, Leu, or Glu;

and wherein said binding polypeptide is displayed on the surface of said bacteriophage.

28. A recombinant bacteriophage expressing exogenous DNA encoding a CEA binding polypeptide having an amino acid sequence comprising:

X₁-X₂-X₃-Cys-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys-X₁₂-X₁₃-X₁₄ (SEQ ID NO:111),

wherein:

X₁ is Asp, or Asn;

X₂ is Trp;

X₃ is Val, Ile, or Met;

X₄ is Asn, or Glu;

X₅ is Leu, Phe, Tyr, Trp, or Ile;

X₆ is Phe, Leu, Asp, Glu, Ile, Ser, Val, or Gly;

X₇ is Lys;

X₈ is Asn, Pro, Gly, Asp, Ala, Ser, His, Met, Val, or Leu;

X₉ is Gln;

X₁₀ is Trp;

X₁₁ is Phe, Thr, Ser, Ala, Asn, Val, His, Ile, Trp, Tyr, Leu, or Glu;

X₁₂ is Asn, or Asp;

X₁₃ is Val, Leu, Ile, Pro, Ala, Gln, Ser, or Met,; and

X₁₄ is Leu, Met, Val, Tyr, Trp, His, Gln, Arg, or Ser.

and wherein said binding polypeptide is displayed on the surface of said bacteriophage.

29. The recombinant bacteriophage according to Claim 26, expressing exogenous DNA encoding an amino acid sequence selected from the group consisting of:

Asn-Trp-Val-Cys-Asn-Leu-Phe-Lys-Asn-Gln-Trp-Phe-Cys-Asn-Ser-Tyr

(SEQ ID NO:4);

Asp-Trp-Val-Cys-Glu-Asn-Lys-Lys-Asp-Gln-Trp-Thr-Cys-Asn-Leu-Leu

(SEQ ID NO:5);

Asn-Trp-Asp-Cys-Met-Phe-Gly-Ala-Glu-Gly-Trp-Ala-Cys-Ser-Pro-Trp

(SEQ ID NO:6);

Asp-Trp-Val-Cys-Glu-Lys-Thr-Thr-Gly-Gly-Tyr-Val-Cys-Gln-Pro-Leu

(SEQ ID NO:7);

Asn-Trp-Phe-Cys-Glu-Met-Ile-Gly-Arg-Gln-Trp-Gly-Cys-Val-Pro-Ser
(SEQ ID NO:8); and
Asp-Trp-Val-Cys-Asn-Phe-Asp-Gln-Gly-Leu-Ala-His-Cys-Phe-Pro-Ser
(SEQ ID NO:9).

30. The recombinant bacteriophage according to Claim 25, expressing exogenous DNA encoding an amino acid sequence selected from the group of sequences depicted in Tables 5, 6, 8, and 9 (SEQ ID NOS:37-109 and 113-151).